

Serology of Brucellosis in Rural Indiana

By S. R. DAMON, Ph.D., C. R. DONHAM, D.V.M., L. M. HUTCHINGS, D. V.M., Ph.D.,
B. T. SIMMS, D.V.M., and JAMES H. STEELE, D.V.M.

REGIONAL LIVESTOCK investigations in conjunction with human epidemiological studies have shown swine and cattle to be major reservoirs of brucellosis in this country. Swine have been primarily implicated in some areas and cattle in others. However, the actual prevalence of infection in the rural human population, a group with a high potential of exposure, and the extent to which it parallels the prevalence in farm animals remains more or less a matter of conjecture.

To obtain a more comprehensive concept of the human-livestock relationships, representatives of several agencies concerned with the problem of brucellosis, meeting in Indianapolis in 1946, suggested a field study of certain Indiana localities. They proposed to investigate areas representative of dairy cattle, swine breeding, and mixed types of animal husbandry by testing farm families and livestock for serologic evidence of infection. It was hoped that the extent of relative infection might then be inferred from the distribution of titers.

Dr. Damon is director of the bureau of laboratories, Indiana State Board of Health; Dr. Donham and Dr. Hutchings, professor of veterinary science and head of the department of veterinary science, Purdue University, respectively; Dr. Simms, chief of the Bureau of Animal Industry, U. S. Department of Agriculture; and Dr. Steele, veterinary director, Communicable Disease Center, Public Health Service.

The project was jointly sponsored by the Bureau of Animal Industry; the department of veterinary science, Purdue University; the Indiana State Board of Health; and the Public Health Service.

Accordingly, the township was selected as a suitable survey unit. The ideal township was considered one where the desired type of animal husbandry was practiced, a high percentage of resident families owned their farms, good lateral roads and a centrally located school or community building existed, no centers of urban population were implicated, and finally, the people were believed to be receptive to such a project. These criteria and recommendations by agencies familiar with the regional agriculture of the State guided the selection of survey areas.

During the winter months of 1946 through 1950 nine townships were surveyed. Blood samples were collected from a major portion of the farm population and livestock and were tested for *Brucella* agglutinins. The average area of the townships was 34 square miles. Surveys were conducted in the winter to avoid conflict with farm activities. Upon completion of all studies, 2,622 persons and 13,373 animals on 930 farms had been tested.

When all data were compiled, there appeared to be little correlation between the human and animal serology. The comparatively small number of human reactors were distributed far more uniformly among the nine townships than were the livestock reactors. These coexisting serologic patterns are presented and discussed in this report.

Plan of the Surveys

Organizing local citizens for the surveys proved an interesting experience in public health tactics. With the county agricultural agent as the pivot, the proposed study was first offered to members of the county medical so-

ciety, then to community leaders who could serve in an organizational capacity. Intensive publicity preceded the actual testing, which was usually completed within 1 month following formal selection of a township for survey. For the most part, local physicians collected the human blood samples, and veterinarians from the Bureau of Animal Industry, U. S. Department of Agriculture, collected the animal blood specimens (1).

Upon completion of each survey, a résumé of the serologic results was reported to the county agent's office for publication in the local newspaper. Positive results of individual human tests were forwarded to physicians designated by the participants at the time their blood specimens were drawn. Results of the animal tests were reported directly to the respective owners. When animal reactors were found on the premises, a State veterinarian or one from the Bureau of Animal Industry returned to discuss possible control methods with the owners.

It should be noted that the incorporation of educational benefits for those persons supplying the raw data and the coordination of both technical and lay facilities of many agencies were integral factors in the program's success.

Serologic Methods

Human serums. Agglutination tests were performed at the Indiana State Board of Health Laboratories. Serums were examined by employing the rapid plate method (Huddleson) as described by Hitchens (2), and a standardized *Brucella abortus* antigen. In performing the tests, serums and antigen were mixed in proportions to give results comparable to those obtained from the examination of serum dilutions of 1:40 and 1:80 by the test tube method. Strongly reacting serums were further diluted to determine titers.

Livestock serums. Specimens collected from animals were examined at the brucellosis testing service laboratory of Purdue University except for those obtained in the Elmore and Steele township surveys, which were tested in a Bureau of Animal Industry mobile laboratory unit.

Serums were examined by using the official

Bureau of Animal Industry plate agglutination technique and *Br. abortus* antigen. In this test, serum and antigen are mixed in proportions to give results comparable to those of the test tube method with serum dilutions of 1:50, 1:100, and 1:200. Examination of swine serums included the 1:25 dilution.

Results

Only individuals 10 years of age or older were examined for *Brucella* agglutinins. Of 2,622 persons, 310 (11.8 percent) came from nearby towns and the remaining 2,312 from rural areas. The former were for the most part included in the Elmore, Steele, and Winfield township surveys. The extent of serologic activity encountered in the total population tested, urban and rural, is summarized in table 1. It was rather surprising to find that only 60 persons (2.3 percent) showed any evidence of agglutinins, and of these only 3 individuals (0.1 percent) 10 years of age or older had a positive titer (4+ reading) of 1:80 or higher. Of the group of 60 persons with agglutinins, only 2 were urban residents and 2 were rural children 10-13 years of age. The remainder were all rural adults.

Of the 2,312 rural persons examined, 16.5 percent were 10-13 years of age, 43.7 percent were males 14 years or older, and 39.8 percent were females 14 years or older. With the exception of Prairie Township, it is estimated that 40-90 percent of the rural population in these age groups were tested in the individual areas. The response in Prairie Township was somewhat less satisfactory. Since the study was designed

Table 1. Extent of serologic activity found in total population¹ tested

Agglutination reading	Number reacting	Percentage of population tested	Percentage of reacting group
1+ at 1:40.....	24	0.9	40.0
>2+ at 1:40.....	33	1.3	55.0
<4+ at 1:80.....			
>4+ at 1:80.....	3	.1	5.0
Total reacting.....	60	2.3	100.0

¹ 2,622 persons, 10 years of age and older.

Indiana townships

where *Brucellosis* surveys were conducted

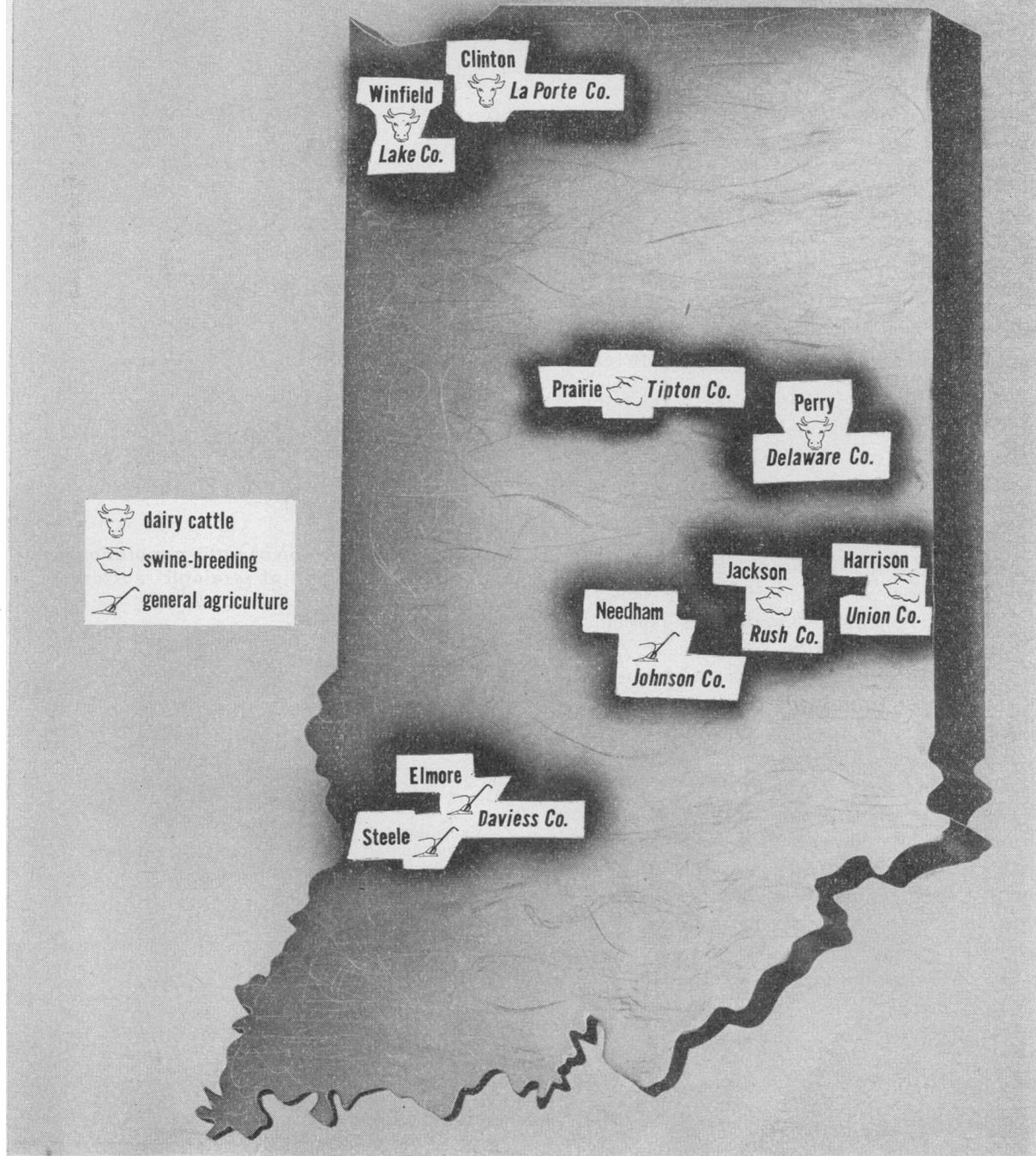


Table 2. Number and percentage of rural persons 14 years and older found with brucellosis titers¹ in the townships

Township	Both sexes			Males			Females		
	Tested	Number	Percent	Tested	Number	Percent	Tested	Number	Percent
<i>Dairy cattle</i>									
Perry.....	361	5	1.4	182	3	1.6	179	2	1.1
Clinton.....	178	3	1.7	96	3	3.1	82	0	0
Winfield.....	223	5	2.2	118	3	2.5	105	2	1.9
<i>Swine breeding</i>									
Harrison.....	169	2	1.2	93	1	1.1	76	1	1.3
Prairie.....	138	2	1.4	66	1	1.5	72	1	1.4
Jackson.....	158	1	.6	82	1	1.2	76	0	0
<i>Mixed husbandry</i>									
Elmore.....	251	5	2.0	138	4	2.9	113	1	.9
Steele.....	196	4	2.0	107	3	2.8	89	1	1.1
Needham.....	256	7	2.7	128	4	3.1	128	3	2.3
Total.....	1,930	34	² 1.8	1,010	23	² 2.3	920	11	² 1.2

¹ At least a 2+ reading with serums diluted 1:40.

² Standard errors are 0.3 percent for both sexes, 0.5 percent for males, and 0.4 percent for females.

primarily to survey the mature farm population, attention is focused upon the prevalence of reactors among the 1,930 persons 14 years and older who were living in the rural areas. The number of reacting individuals in this group is given for each township in table 2. Based on all nine township samplings, 1.8 percent of the persons tested displayed some positive titer. Among the males, 2.3 percent reacted. Of the females, 1.2 percent reacted. This difference of 1.1 percent was found to be of only slight statistical significance.

A tabulation of information obtained concurrently with the blood testing indicated more than 80 percent of the adult males, approximately 70 percent of the children 10-13 years of age, but only 54 percent of the adult females with histories of direct animal contact. All those rural persons showing a serologic titer, with four exceptions, claimed animal contact. The four exceptions, however, did drink raw milk. Information on the consumption of raw milk by persons included in the surveys was incomplete.

Prevalence in Cattle

The majority of animals tested were cattle, 7,918 altogether. At least 60-80 percent of the

adult cattle were surveyed in each township except Prairie where the percentage was somewhat less. At the time of drawing the blood samples, field crews obtained information re-

Table 3. Composition of cattle population tested and distribution of serologic reactors¹

Group	Number tested	Percentage of total population	Reactors	
			Number	Percentage of group
All cattle.....	7,918	100.0	543	6.9
Vaccinated.....	788	10.0	179	22.7
Unvaccinated.....	6,999	88.4	347	5.0
Unknown.....	131	1.6	17	13.0
Unvaccinated cattle:				
Males.....	404	5.1	6	1.5
Under 1 year... 65		.8	0	0
1 year or more.. 339		4.3	6	1.8
Females.....	6,595	83.3	341	5.2
Under 1 year... 348		4.4	7	2.0
1-1.9 years..... 823		10.4	9	1.1
2 years or more.. 5,424		68.5	325	6.0

¹ At least a 4+ agglutination reading in 1:100 dilution.

garding age, sex, and prior use of *Brucella* vaccine. These data are summarized in table 3 along with the total number (543) and percentage (6.9) of reactors found.

A bovine reactor in this study is defined as one whose serum at a dilution level of 1:100 or higher completely agglutinated the standard antigen. Those animals giving a partial reaction at this level or a complete reaction only in the 1:50 dilution are designated as suspects. Data are tabulated separately for all cattle and for only unvaccinated cattle. These two analyses are included to facilitate interpretation of results and their subsequent comparison with findings reported from other areas.

In table 4 an area breakdown is given for the number and percentage of cattle reactors encountered among the total number tested, among the unvaccinated animals only, and among the unvaccinated mature females. The latter group contributed 93.7 percent of all unvaccinated cattle reactors. In addition to the positive reactors, 645 (8.1 percent) of the total cattle tested were found to be suspects. When the results from testing vaccinated ani-

imals were excluded from the data, this number was reduced to 440 (5.6 percent).

Final tabulations showed that 10 percent of the tested animals, or one-third (32.8 percent) of all reactors, had been previously vaccinated either as calves or as adults. Of the vaccinated cattle, 22.7 percent were reactors and an equal number were suspects. A certain number of the vaccinated animals which were reactors had been immunized just before the time of testing. The inclusion of results from testing the vaccinated animals increased the percentage of reactors approximately 2 percent, from 5.0 to 6.9.

Inspection of the data in table 4 indicates the variation in the percentage of cattle reactors—and probable infection—to be found in the different localities. When the data were tested for statistical significance, the percentage of reactors encountered in the dairy-cattle townships was found to be significantly different from that found in each of the other agricultural categories. Thus, brucellosis in cattle appears to be much less a problem in some areas than in others—less especially in Steele Town-

Table 4. Cattle reactors¹ found in each township

Township	All cattle			Unvaccinated cattle					
	Tested	Reactors	Percent	Total			Mature females ²		
				Tested	Reactors	Percent	Tested	Reactors	Percent
<i>Dairy cattle</i>									
Perry.....	1, 178	105	8. 9	989	55	5. 6	744	53	7. 1
Clinton.....	1, 039	66	6. 4	774	30	3. 9	605	30	5. 0
Winfield.....	1, 295	173	13. 4	1, 112	139	12. 5	892	129	14. 5
<i>Swine breeding</i>									
Harrison.....	601	45	7. 5	505	9	1. 8	388	9	2. 3
Prairie.....	654	25	3. 8	631	17	2. 7	500	12	2. 4
Jackson.....	566	24	4. 2	563	21	3. 7	437	21	4. 8
<i>Mixed husbandry</i>									
Elmore.....	955	14	1. 5	955	14	1. 5	739	14	1. 9
Steele.....	610	12	2. 0	591	3	. 5	476	3	. 6
Needham.....	1, 020	79	7. 7	879	59	6. 7	643	54	8. 4
Total.....	7, 918	543	³ 6. 9	6, 999	347	³ 5. 0	5, 424	325	³ 6. 0

¹ At least a 4+ agglutination reading in the 1:100 dilution.

² 2 years of age or older.

³ Standard errors are 0.3 percent for each of the three groups.

Table 5. Sex distribution of swine reactors¹ found in each township

Township	All swine			Males			Females		
	Tested	Reactors	Percent	Tested	Reactors	Percent	Tested	Reactors	Percent
<i>Dairy cattle</i>									
Perry.....	574	6	1.0	48	1	2.1	526	5	1.0
Clinton.....	301	3	1.0	17	0	0.0	284	3	1.1
Winfield.....	123	5	4.1	8	0	0.0	115	5	4.3
<i>Swine breeding</i>									
Harrison.....	822	20	2.4	53	1	1.9	769	19	2.5
Prairie.....	403	4	1.0	25	2	8.0	378	2	.5
Jackson.....	1,118	89	8.0	59	2	3.4	1,059	87	8.2
<i>Mixed husbandry</i>									
Elmore.....	397	5	1.3	23	0	0.0	374	5	1.3
Steele.....	393	31	7.9	26	0	0.0	367	31	8.4
Needham.....	271	14	5.2	20	4	20.0	251	10	4.0
Total.....	4,402	177	² 4.0	279	10	² 3.6	4,123	167	² 4.0

¹ At least a 4+ agglutination reading in the 1:50 dilution.

² Standard errors are 0.3 percent for all swine, 1.1 percent for males, and 0.3 percent for females.

ship than in Winfield Township, which is located in the Chicago milkshed.

Prevalence in Swine

For the purpose of this study, a swine reactor is considered one whose serum agglutinated *Brucella* antigen completely in a serum dilution of 1:50 or higher. If merely a partial reaction occurred at this level or if the reaction was evident only at the 1:25 dilution level, the animal is termed a suspect.

In all 9 surveys 4,402 swine were tested, 279 males (6.3 percent) and 4,123 females (93.7 percent). Approximately 50-80 percent of the total mature swine in each area were surveyed.

Four percent of the hogs (177) were found to be reactors and 417 (9.5 percent) were suspects; 3,808 (86.5 percent) were negative. The number of reactors found in each township is given in table 5. No statistical significance was noted in the percentage differences between the sexes. As with cattle, the percentage of swine reactors in the dairy-cattle areas was found to be significantly different from that of the swine-breeding areas as well as from that of the general agricultural category.

The presentation of serologic data does not entirely dispose of the question of prevalence of

Table 6. Swine suspects¹ found on farms having swine reactors²

Township	Total swine with agglutinins		Suspects found on farms having swine reactors	Reactors plus significant suspects ³	
	Reactors	Suspects		Number	Percent
<i>Dairy cattle</i>					
Perry.....	6	43	5	11	1.9
Clinton.....	3	16	3	6	2.0
Winfield.....	5	10	4	9	7.3
<i>Swine breeding</i>					
Harrison.....	20	49	23	43	5.2
Prairie.....	4	22	6	10	2.5
Jackson.....	89	127	79	168	15.0
<i>Mixed husbandry</i>					
Elmore.....	5	32	5	10	2.5
Steele.....	31	62	49	80	20.4
Needham.....	14	56	26	40	14.8
Total.....	177	417	200	377	8.6

¹ Less than a 4+ agglutination reading in the 1:50 dilution and agglutination at the 1:25 dilution level.

² At least a 4+ agglutination reading in the 1:50 dilution.

³ Suspects found on farms having swine reactors.

infection in this species. Control programs based on the eradication of only those animals showing a positive titer of 1:50 or higher often have failed. A certain percentage of animals manifesting lower titers will actually harbor *Brucella* and when retained may subsequently reinfect the herd, while indiscriminate disposal of all hogs reacting at this lower serum dilution level of 1:25 may result in the condemnation of many uninfected animals (3).

For these reasons, it is felt that whereas the higher titer is the more reliable index to infection in the random individual, the lower titer is more satisfactory for indicating infection in animals from herds where there are swine showing higher titers. Accordingly, the data obtained in the township surveys are further analyzed to show the number and percentage of suspects which were found on farms with swine showing higher titers (table 6). Approximately half the swine designated as suspects came from herds with swine having higher titers. When this number (200) is added to the 177 positive reactors, 377 or 8.6 percent of the total swine tested might well be considered as possibly harboring the infective organism.

Other Farm Animals

Other farm animals tested numbered 1,053: 671 sheep, 354 horses and mules, and 28 goats. None of the goats reacted. Of the sheep, 5 (0.7 percent) were positive reactors and 14 (2.1 percent) were suspicious. Of the horses and mules, 9 (2.5 percent) reacted positively, while 43 (12.1 percent) were considered suspects. The terms "reactor" and "suspect" are defined the same as previously for cattle.

Farms with Reactors

In obtaining specimens from the livestock, Bureau of Animal Industry field crews visited 930 farms. It is estimated that the livestock on approximately 75 percent of the premises in the nine areas were surveyed. One or more animal reactors were found on 260 (28.0 percent) of the farms. Nine farms (1.0 percent) harbored horse or mule reactors and 5 (0.5 percent) harbored sheep reactors. There was none with reacting goats. The township distribution of premises with cattle and swine reactors is given

in table 7. It can be seen from these figures that the exclusion of data collected from farms having vaccinated animals reduces approximately 5 percent the number of farms with cattle reactors.

The infrequent occurrence of both cattle and swine reactors on the same premises is of interest. Considering only those farms having all unvaccinated animals (849), cattle reactors were found on 135 (15.9 percent). On the other hand, reactor or suspect swine, or both, were detected on only 35 of the 135 premises, although nearly all farms having a cattle reactor also had swine tested on the premises. Actually, only 6 (0.7 percent) of the 849 farms possessed both a cattle reactor and a swine reactor. The other 29 farms had only swine suspects.

In some townships a few large, heavily stocked farms accounted for a large percentage of the reactors. These data have not been presented because total animal counts were not obtained on all farms. However, there definitely appeared to be a direct relationship between the number of animals tested on a farm and the percentage of farms with animal reactors. When results from those farms which had vaccinated cattle on their premises were excluded from the data and when the remaining farms were tabulated according to the number of animals tested on each and a grouped frequency distribution table made, it was found that the number of farms with reactors increased with the indicated increase in herd size. Only 0.3 percent of the farms having 1-5 animals tested had swine reactors and 4.8 percent had cattle reactors whereas 27.4 percent of the farms having over 25 animals tested had swine reactors and 31 percent had cattle reactors. The other step intervals showed gradations between these two extremes. These results are consistent with those of many investigators.

In table 8, the proportion of farms with reactors and the percentage of reactors found in the various species have been related with respect to the three types of agricultural areas.

Discussion

Despite the fact that percentages of cattle and swine reactors varied from one township to

another, the percentages of human reactors remained fairly constant. An inspection of the data does not indicate any direct correlation between prevalence of animal and human agglutination titers.

In attempting an explanation for these results, consideration must be given to several possibly significant factors. Serology itself is a cumulative index of past experiences which differ fundamentally in the relatively stable rural population and in the constantly changing livestock. Furthermore, titers resulting from exposure to the animals surveyed cannot be differentiated from titers resulting from contact with previously owned animals. Although there may exist a direct correlation between the incidence of new infections in human and animal populations, this study would not necessarily reveal such a relationship.

Animal contacts are largely determined by regional agricultural practices and vary from one locality to another or even from farm to farm. Virulence of *Brucella* strains are different. Consequently, a farm with many in-

fectured swine or beef cattle raised for commercial markets may not constitute the exposure provided by a single milk cow, hog, or possibly a riding horse infected with a more virulent strain of *Brucella*. It is possible that these interrelated factors may have operated to produce fairly uniform serologic patterns in man and not in the livestock.

It should be further mentioned that a sampling error in the human testing may exist. Since the blood specimens were collected at a designated testing station in each township, persons confined to their living quarters were not surveyed. This would mean that any acutely ill brucellosis cases were not tested. However, in view of the variety of diseases occurring in a population group during the winter months, it does not seem very probable that the ratio of acute brucellosis cases to the composite of other disease would have been so abnormally high as to alter the findings significantly. Inspection of the data does not indicate an unduly poor response on the part of any age or sex group to account for the small

Table 7. Number and percentage of surveyed farms with animal reactors

Township	Farms surveyed	Farms with reactors ¹							
		All species		Cattle				Swine	
				Total		Unvaccinated			
		Number	Percent	Number	Percent	Number	Percent ²	Number	Percent
<i>Dairy cattle</i>									
Perry.....	137	43	31.4	43	31.4	23	20.7	4	2.9
Clinton.....	79	27	34.2	25	31.6	10	16.9	2	2.5
Winfield.....	88	50	56.8	48	54.5	34	45.9	5	5.7
<i>Swine breeding</i>									
Harrison.....	63	22	34.9	11	17.5	8	13.3	11	17.5
Prairie.....	92	18	19.6	15	16.3	12	13.5	4	4.3
Jackson.....	75	33	44.0	12	16.0	11	14.9	19	25.3
<i>Mixed husbandry</i>									
Elmore.....	161	15	9.3	11	6.8	11	6.8	5	3.1
Steele.....	115	10	8.7	4	3.5	3	2.7	6	5.2
Needham.....	120	42	35.0	31	25.8	23	21.1	8	6.7
Total.....	930	260	³ 28.0	200	³ 21.5	135	³ 15.9	64	³ 6.9

¹ Swine readings are 4+ agglutination in 1:50 dilution; other animals, 4+ in 1:100 dilution.

² Percentage of those farms which had no vaccinated animals on premises on which cattle reactors were found.

³ Standard errors for each group, reading from left to right, are 1.5 percent, 1.4 percent, 1.2 percent, and 0.8 percent.

Table 8. Summary of percentage of incidences of brucellosis serologic reactors and percentage of farms with reactors according to agricultural category of townships

Agricultural category	Human	Cattle ¹	Swine	Equine	Sheep	Farms with reactors		
						All species	Unvaccinated cattle	Swine
Dairy cattle.....	1.7	7.8	1.4	3.4	0.8	39.5	27.5	3.6
Swine breeding.....	1.1	2.8	4.8	3.7	.7	31.7	13.9	14.8
General agriculture.....	2.3	3.1	4.7	1.3	0	16.9	9.7	4.8
Total.....	1.8	5.0	4.0	2.5	.7	28.0	15.9	6.9

¹ Unvaccinated.

number of positive reactors. There is a possibility, however, that the seasonal nature of the surveys did not take into consideration any peak occurrences of high titers which may have prevailed in Indiana during certain months.

Despite the many variables which enter into a study of this type, the importance of obtaining serologic patterns as an indication of the distribution and relationship of human and animal brucellosis should not be minimized. While the foregoing data are not conclusive with regard to the effect of animal brucellosis upon the spread of human infection, they are presented as evidence of the wide regional variation which may be found in the character of the animal reservoirs of brucellosis and further as an indication of serologic patterns which may possibly coexist in rural areas such as the ones surveyed.

Summary

Serologic surveys for the relative prevalence of *Brucella* agglutinins in livestock and farm families were conducted in nine rural townships representing the dairy cattle, swine breeding, and general agricultural areas of Indiana.

Less than 2 percent of 2,622 residents 14 years of age or older reacted positively to some degree. The percentage of reactors was approximately the same in each of the townships.

Of 13,373 farm animals, approximately 6 percent were found to be reactors. Of the 930 farms surveyed, 28.0 percent were found to harbor livestock reactors. Four percent of the swine population, 6.9 percent of the cattle, 2.5

percent of the horses and mules, and 0.7 percent of the sheep were reactors. Ten percent of all cattle had been previously vaccinated. When results of testing these animals were excluded from the data, 5.0 percent of the cattle were reactors. These percentages varied considerably in the different townships.

If those swine suspects found on farms having hogs with higher titers were added to the number of actual reactors, then 8.6 percent of the swine population surveyed might be considered as possibly *Brucella*-infected.

ACKNOWLEDGMENTS

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